

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

IMPACT OF PREGNANCY ON PROGNOSIS OF DIFFERENTIATED THYROID CANCER: CLINICAL AND MOLECULAR FEATURES.

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/153341> since

Published version:

DOI:10.1530/EJE-13-0903

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



UNIVERSITÀ DEGLI STUDI DI TORINO

This is not the definitive version of record of this article.

This manuscript has been accepted for publication in Journal of *European Journal of Endocrinology* but the version presented here has not yet been copy edited, formatted or proofed. Consequently, Bioscientifica accepts no responsibility for any errors or omissions it may contain. The definitive version is now freely available at *10.1530/EJE-13-0903, 2014*.

IMPACT OF PREGNANCY ON PROGNOSIS OF DIFFERENTIATED THYROID CANCER: CLINICAL AND MOLECULAR FEATURES

Ilaria Messuti, Stefania Corvisieri, Francesca Bardesono, Ida Rapa¹, Jessica Giorcelli¹,
Riccardo Pellerito², Marco Volante¹, Fabio Orlandi

Endocrine Unit, Department of Oncology, University of Turin, Presidio Sanitario

Gradenigo, Turin, Italy

1 Pathology Unit, Department of Oncology at San Luigi Hospital, University of Turin,

Orbassano, Turin, Italy

2 Nuclear Medicine Unit, Mauriziano Hospital, Turin, Italy

(I Messuti and S Corvisieri equally contributed to this work)

CORRESPONDING AUTHOR

Fabio Orlandi

Corso Regina Margherita 10, Torino; fabio.orlandi@unito.it

KEY WORDS

pregnancy, thyroid cancer, estrogen receptor, DTC outcome

Manuscript: 3739 words

33 **ABSTRACT**

34 **Objective:** Differentiated Thyroid Cancer (DTC) commonly occurs in women of child-
35 bearing age and represents the second most frequent tumor diagnosed during pregnancy
36 only behind breast cancer. It is possible that associated physiological changes could
37 favour tumor development and growth. However, few data are available about the
38 outcome of DTC related to pregnancy, leading to conflicting results. **Methods:** 340
39 patients with DTC <45 years old were retrospectively studied. Patients were divided into
40 three groups according to the time of tumor diagnosis respect of pregnancy. Group 1:
41 diagnosis of DTC at least 2 years after delivery, Group 2: diagnosis during pregnancy or
42 within the second year after delivery, Group 3: nulliparous patients at the time of diagnosis.
43 We evaluated clinical outcome and immunohistochemical expression of estrogen receptor
44 α (ER α), estrogen receptor β (ER β), progesterone receptor (PGR) and aromatase. We
45 also analysed the gene expression of NIS and the prevalence of BRAF V600E mutations.
46 **Results:** Persistence/recurrence of disease was significantly higher in group 2 patients
47 than control groups (p: 0.023). No significant differences were observed in other clinical
48 parameters. Furthermore no difference among the groups were recorded about ER
49 pattern, NIS expression and BRAF mutations. **Conclusions:** Persistence/recurrence of
50 DTC is significantly higher in pregnant patients, suggesting that pregnancy could really
51 exert a negative prognostic role in patients with DTC. The underlying mechanisms are not
52 yet clarified and further studies are required. Our results suggest that a more careful follow
53 up is needed when diagnosis of DTC occurs during pregnancy or shortly after.

54

55 **Abbreviations**

56 DTC: differentiated thyroid cancer; rhTSH: Recombinant human thyroid stimulating
57 hormone; Tg: tireoglobuline; S-Tg: tireoglobuline measured under suppressive therapy
58 with I-T4; A-Tg: tireoglobuline measured at ablation with I-131; rhTSH-Tg: tireoglobuline
59 measured after stimulation with rhTSH; AbTg: Anti-Tireoglobuline Antibody; MTS:
60 metastasis; ER: estrogen receptor; AR: androgen receptor; PGR: progesterone receptor;
61 RAI; radioiodine ablations with I-131; NIS: Na-I symporter

62

63 **INTRODUCTION**

64 Differentiated Thyroid Cancer (DTC) is a relatively rare neoplasia. It represents 3.6% of all
65 malignant tumors in the United States (SEER Cancer Statistics Review, National Cancer
66 Institute Surveillance, Epidemiology, and End Results; 1975–2005. Available from
67 <http://seer.cancer.gov>) and it is generally characterized by good prognosis. Consequently,
68 studies evaluating the prognosis of this tumor have to consider a wide number of cases
69 and a long-term follow-up to highlight differences in survival or disease recurrence rate.
70 The majority of relapses usually occurs within 5 years from the initial treatment, and only
71 sporadic cases have been subsequently documented (1, 2). Despite the low incidence, in
72 the USA DTC represents the second most frequent diagnosed tumor during pregnancy,
73 only after breast cancer. In women of child-bearing age about 10% of thyroid carcinomas
74 is diagnosed during pregnancy or early after delivery (SEER Cancer Statistics Review,
75 National Cancer Institute Surveillance, Epidemiology, and End Results; 1975–2005.
76 Available from <http://seer.cancer.gov>). These findings have led to hypothesize that during
77 this period the presence of several physiologic changes, such as hormonal secretion,
78 growth factors and negative iodine balance, could create a favourable environment for the
79 development and growth of tumors.

80 However, only few studies about the outcome of DTC related to pregnancy have been
81 published. A recent review (3) reports that pregnancy is not generally described in
82 literature as a determining condition for prognosis of DTC, neither in terms of DTC-related
83 death (4), nor of overall survival (5, 6) .

84 Nevertheless, these findings are in contrast with the more recent study published by
85 Vannucchi et al. (7), who reported that DTC in pregnant women had a significant increase
86 of persistent/recurrent disease than those in non pregnant patients. Since the parameters
87 and the methodology in each study were very different, their results were not easily
88 comparable. In fact, the studies conducted by Yasmeen et al. (5) and Herzon et al. (6),
89 focused mainly on the overall survival, while the study of Moosa and Mazzaferri (4) had
90 DTC-related death and disease recurrence, evaluated by biopsy or by ¹³¹I uptake in
91 distant sites, as primary focus. On the contrary, Vannucchi et al. (7) have evaluated
92 persistent/recurrent DTC through more sensitive tests, such as basal and stimulated Tg
93 levels after exogenous TSH injection (rhTSH, Thyrogen[®], Genzyme Corporation, Sanofi
94 Company, Cambridge, MA) which have not been used in the other studies and may - at
95 least partially - explain the different conclusions.

96 Moreover, Vannucchi et al. (7) observed a significantly higher immunohistochemical
97 expression of the Estrogen Receptor α (ER α) in tumors from pregnant women compared
98 to the control groups. With special reference to hormone receptor expression in thyroid
99 tumors, a recent study (8) on the immunoistochemical expression of estrogen and
100 androgen receptors (AR) in DTC showed that ER α was acquired or increased in tumor
101 samples as compared to the corresponding normal tissue, whereas AR and ER β
102 expression was decreased in tumors compared with the surrounding normal tissue. These
103 patterns appeared also to be associated with the clinical behaviour, being the high

104 expression of ER α and AR and the low expression of Er β associated with a more
105 aggressive phenotype.

106 In such a controversial situation, we therefore designed the present study to characterize
107 at clinical, phenotypical and molecular levels DTC cases in pregnancy as compared to
108 matched control groups.

109

110 **MATERIAL AND METHODS**

111 **Patients**

112 We retrospectively evaluated more than 1200 medical records of patients with DTC treated
113 and followed up from 2001 to 2011 at the Nuclear Medicine Department of Mauriziano
114 Hospital, which covers up to 80% of all radioiodine ablations with I-131 (RAI) performed in
115 the Piedmont region. This allowed us to obtain an extremely homogeneous population,
116 representative of DTC epidemiology in the fertile women population.

117 Among them, 340 women were selected according to the following inclusion criteria:

- 118 • Age \leq 45 years at the time of surgery
- 119 • Total thyroidectomy
- 120 • I-131 radioiodine ablation
- 121 • L-T4 TSH-suppressive therapy (TSH \leq 0.1 mU/l) (9)
- 122 • Follow up \geq 1 year
- 123 • rhTSH test during follow up or persistent disease (S-Tg > 2 ng/ml)

124

125 Patients were divided into three groups according to the time of diagnosis of DTC respect
126 to pregnancy. Group 1 included women (n=152, median age 40, range 25-45) with DTC

127 diagnosis at least 2 years after delivery. Group 2 included women (n=38, median age 35,
128 range 26-41) with diagnosis of DTC during pregnancy or within two years after delivery.
129 Group 3 included nulliparous patients at the time of diagnosis (n=150, median age 30,
130 range 15-45).

131 Tumors were classified following the World Health Organization classification (10), staged
132 according to the 6th edition of TNM staging (American Joint Committee on Cancer, AJCC).
133 (11) and classified as Low and High Risk according to the European Consensus
134 Statement criteria (9). ETA guidelines divide patients into three groups: Very Low, Low and
135 High Risk but the first group was not represented in our series because it includes patients
136 with no indications for RAI .

137 Remission or persistent/recurrent disease were defined according to the European and
138 American guidelines for the management of DTC (9, 12):

- 139 ▪ REMISSION: S-Tg and rh-TSH-Tg < 0,6 mcg/l, negative AbTg and
140 normal neck ultrasound.
- 141 ▪ PERSISTENT/RECURRENT disease at least one of the following criteria:
 - 142 - S-Tg > 2 µg/L
 - 143 - rh-TSH-Tg > 2 µg/L
 - 144 - Persistence of AbTg > 4 years with a trend to increase (an increasing
145 antibody production or newly antibodies appearance as a consequence of an
146 increased of autoantigen production) (13, 14)
 - 147 - Neck or distant MTS
 - 148 - Radioiodine uptake outside thyroid bed

149

150 Serum Tg levels were measured during L-T4 withdrawal, immediately before RAI and after
151 12 months of L-T4 suppressive therapy. Then, patients received one injection of rh-TSH
152 (0.9 mg i.m., Thyrogen[®], Genzyme Corporation, Sanofi Company, Cambridge, MA) for two
153 consecutive days; serum samples for TSH and Tg measurements were collected on days
154 0 (before first rh-TSH administration), 3 and 4. Neck ultrasonography was performed 6 and
155 12 months after RAI. TSH levels were evaluated using a chemiluminescent immunoassay
156 (Access Immunoassay Systems, Beckman Coulter[®], Inc.), Tg levels were determined
157 using chemiluminescent immunoassay (Access Immunoassay Systems, Beckman
158 Coulter[®]), with a functional sensitivity of 0.6 µg/L; Ab-Tg antibodies were detected with
159 chemiluminescent immunoassay (Access Immunoassay Systems, Beckman Coulter[®],
160 Inc.). Based on Tg assay, we considered 0.6 µg/L as the cut-off value between
161 undetectable and measurable Tg levels, according to Mazzaferri *et al.* (1)

162

163 **Immunohistochemical analysis of hormone receptors and aromatase in tumor** 164 **tissues**

165 Immunohistochemical evaluation of estrogen receptor α (ER α), estrogen receptor β (ER β),
166 progesterone receptor (PGR) and aromatase were performed in 37 histological specimens
167 selected from the three different groups (12 of group 1, 10 of group 2, 15 of group 3).
168 Cases for immunohistochemical analysis were blinded selected to obtain 3 groups
169 homogenous in terms of age and stage, regardless of the outcome.

170 Immunohistochemical analyses were carried out on paraffin-embedded tissue sections of
171 5 µm, after dewaxing, dehydration in alcohol and rehydration in PBS pH 7,5. Endogenous
172 peroxidase block was performed by immersion of the slides in 0,3% solution of methanol
173 and hydrogen peroxide for 15 minutes. Then sections were incubated with the following
174 monoclonal primary antibodies: ER α (clone 1D5, dilution 1:300, Dako, Glostrup, DK), ER β

175 (clone PPG5/10, dilution 1:50, Dako), PGR (clone 636, dilution 1:300, Dako), and
176 Aromatase (clone mca2077s, dilution 1:50, Serotec, Kidlington, UK). A biotin-free, dextran
177 chain-based detection system (EnVision, Dako) and diaminobenzidine as the chromogen
178 were used according to standard protocols. All markers were assessed in tumoral and
179 peritumoral tissue using H-score evaluation, which takes into account both quantitative
180 and qualitative expression with a 0-300 range scale.

181

182 **Molecular analysis**

183 ***Nucleic acids isolation.*** Genomic DNA was isolated from formalin-fixed, paraffin-
184 embedded tissues using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). RNA was
185 isolated from paraffin embedded material using the high pure RNA paraffin kit (Roche,
186 Mannheim, Germany) following the manufacturer's instructions. The quantity of isolated
187 DNA and RNA was assessed using a Biophotometer (Eppendorf, Hamburg, Germany).

188 ***BRAF point mutation analysis.*** The presence of *BRAF* point mutation (V600E) was
189 analysed using pyrosequencing and PCR primers following previously published protocols
190 (15). PCR amplification for the pyrosequencing assay was performed according to
191 standard protocols. The amplicons were mixed with sequencing primers and sequencing
192 was performed using a PyroGold reagent kit (Biotage AB) according to the manufacturer's
193 protocol. Results were analyzed using the PSQ-96 MA 2.0.2 software (Biotage).

194 ***Quantitative real time PCR for the sodium/iodide symporter.***

195 Relative cDNA quantitation of the sodium/iodide symporter (NIS) and an internal reference
196 gene (β -actin) were done using a fluorescence-based real-time detection method [ABI
197 PRISM 7900 Sequence Detection System (Taqman); Applied Biosystems/Life
198 technologies, Foster City, CA]. Beta-actin primers and probe were previously published
199 (16), whereas for NIS the TaqMan gene expression assay 20X (SLC5A5

200 Hs00166567_m1, Applied Biosystems) was used according to manufacturer's instructions.
201 The PCR reaction mixture consisted 1,200 nmol/L of each primer, 200 nmol/L probe, 200
202 nmol/L each of dATP, dCTP, dGTP, dTTP, 3.5 mmol/L MgCl₂, and 1x Taqman Universal
203 PCR Master mix to a final volume of 20 μ L (all reagents were from PE Applied
204 Biosystems,). Cycling conditions were 50°C for 2 minutes, 95°C for 10 minutes, followed
205 by 46 cycles at 95°C for 15 seconds and 60°C for 1 minute. To analyze target gene
206 expression in individual tumors, the relative gene expression levels were expressed as
207 ratios (differences between the C_t values) between 2 absolute measurements (genes of
208 interest/internal reference gene). Then, the $\Delta\Delta C_t$ values were calculated subtracting ΔC_t
209 values of each case to the value of the normal sample expression, and converting the ratio
210 by the $2^{-\Delta\Delta C_t}$ formula; cases were considered of low or high expression according to the
211 median expression level obtained.

212

213 **Statistical analysis**

214 The clinical (age, outcome, number of treatments, ablation-Tg levels, High/Low risk
215 classification) and pathological/molecular features (histology, pTNM stage, hormone
216 receptor expression, NIS gene expression and BRAF mutation status) were compared
217 among the three groups of patients by using the χ^2 test for dichotomic variables and the
218 Mann Whitney and Kruskal-Wallis tests for continuous variables, as appropriate. The
219 reciprocal correlation among immunohistochemical markers was evaluated using the
220 Spearman's test. Statistical significance was defined as $p < 0,05$.

221 A logistic multivariable analysis was performed. Dependent dichotomous variable was
222 tumor persistence/recurrence (1) or remission (0). Age, T, N and multifocality of primary
223 tumor and pregnancy (DTC diagnosis during pregnancy or within 2 years after delivery: 1;

224 Other groups: 0) were the independent variables. All these analyses were performed using
225 STATISTICA for Windows Ver. 8.0.

226

227

228 **RESULTS**

229 Clinical, biochemical, histopathological and molecular parameters in the three groups are
230 reported in Table 1.

No significant differences were noticed in the number of treatments for achieving clinical remission, in the tumor size or extrathyroidal invasion, in the lymphnodal metastatic involvement at diagnosis, in histology and in High Risk/Low Risk classification of patients according to the ETA guidelines (9).

231 Clinical remission was obtained in 150/152 patients (98.7%) of group 1, 34/38 patients
232 (89.5%) of group 2 and in 143/150 patients (95.3%) of group 3. Persistent/recurrent
233 disease was observed in 2/152 patients (1.3%) of group 1, 4/38 patients (10.5%) of group
234 2 and in 7/150 patients (4.7%) of group 3. Our results showed a significant difference (χ^2 :
235 7.532, $P= 0.023$) in the outcome among the three groups, with a greater percentage of
236 persistent disease in group 2 than in group 1 and 3. Group 1 and 3 did not show any
237 significant difference. Only 4/38 patients in group 2 had cytological diagnosis while
238 pregnant. They underwent thyroidectomy in the early postpartum period, achieving clinical
239 remission, showing that the surgical delay of few months was not a factor that could
240 influence the worst outcome of group 2.

241 As regards the expression of hormone receptors (Figure 1), the percentage of intratumoral
242 and peritumoral expression of ER α in the 37 histological samples were globally low, with
243 no detection of significant differences between the groups ($P=0.96$). ER β showed a high
244 expression in the peritumoral tissue in a large number of cases, while in tumoral tissue its

245 expression was quite variable, similarly in the three groups ($P=0.82$). PGR expression was
246 mostly negative in peritumoral tissue, while it was quite variable in tumoral tissue, in a
247 similar way in the three groups ($P=0.41$). A significant correlation was observed in tumor
248 tissue between ER α and PGR (Spearman's R value: $R=0.49$; $P=0.002$). Aromatase was
249 negative both on peritumoral and tumoral tissue in all the samples analyzed. BRAF V600E
250 mutation, known as a negative prognostic factor (18), was detected in 25% in group 1,
251 44,4% in group 2 and 60% in group 3 (average of whole samples= 43%). The difference
252 was not statistically significant ($P=0.191$) showing that the worst outcome observed in
253 patients of group 2 is independent from BRAF mutation. However, BRAF was mutated in
254 100% of patients with persistence of disease and in 37,5% of patients in remission,
255 irrespective of the group.

256 NIS gene expression levels were also not different in the three groups ($P=0.82$) nor
257 associated to BRAF mutation status ($P=0.55$).

258 Logistic multivariable analysis performed on the whole population (thus excluding
259 molecular analyses) showed pregnancy (Group 2) as the unique independent variable for
260 persistent/recurrent DTC prediction. The relative risk (RR) was 1.12 (95% CI 1.02-1.22;
261 $p=0.02$). Age, T, N and multifocality of the primary tumor did not enter the model.

262 In the 37 patients with molecular and immunohistochemical data available, BRAF mutation
263 and low NIS expression were strong independent predictors of persistence/recurrence of
264 DTC (Table 2), whereas ER α and PGR did not enter the model. Pregnancy and ER β
265 positivity were of borderline statistical significance. .

266 Power analysis was performed grouping the entire population into patients with DTC
267 during or within 2 years after delivery (38 subjects) vs all other patients (302 subjects), with
268 values of 79% and 87%,. by two-sided and one-sided tests, respectively.

269

270 **DISCUSSION**

271 Thyroid cancer discovered during pregnancy represents a challenge for the clinicians
272 because, at present, there are still no reliable data available supporting a specific
273 management of pregnancy-associated DTCs. Currently pregnant patients with a
274 cytologically suspicious thyroid nodule for DTC do not require surgery during pregnancy
275 except in cases of rapid nodular growth and/or the appearance of lymph node metastases
276 (19).

277 Most studies showed that pregnancy did not worsen the prognosis of DTC. In four studies,
278 the prognosis of women with DTC diagnosed either during pregnancy or within the first
279 postpartum period was compared to that of women diagnosed at another time as controls.
280 In three of these works (4-6), no difference was found in DTC prognosis between pregnant
281 women and control groups. However, in the fourth study (7), Vannucchi et al. reported a
282 significant worse outcome in pregnant patients. As a matter of fact, they observed 60% of
283 recurrent/persistent disease in pregnant women (group 2) vs 4.2% in women with DTC
284 diagnosed more than 1 year after delivery (group 1) and 13.1% in nulliparous patients
285 (group 3). Moreover, a higher expression of ER α in tumor samples of pregnant women
286 was reported.

287 In order to verify these conflicting results, we selected a homogeneous population, dividing
288 patients into three groups according to the criteria adopted by Vannucchi et al.. We
289 extended group 2 to women with DTC diagnosis within 2 years after delivery instead of 1
290 year, arbitrarily assuming that in tumors with low biological aggressiveness, such as DTC,
291 pregnancy-induced hyperestrogenism may exert its tissue activity in a longer period. At our
292 knowledge no published data are available on this issue. Moreover, in our population the
293 rate of persistent/recurrent disease in patients diagnosed within 1 year or between 1 and 2
294 years after delivery was very similar (9,5% - 2/21 cases - and 11,7% - 2/17 cases –
295 respectively). On the contrary, all the patients (14/14) diagnosed between 2 and 3 years

after delivery displayed clinical remission.

Consistent with the data reported by Vannucchi et al, we confirmed a significant correlation between pregnancy and a worse outcome of DTC ($p= 0,023$), representing the unique independent variable for persistent/recurrent disease prediction.

Indeed, thyroid cancer diagnosed during pregnancy (group 2) was found to be significantly associated with persistence or relapse of DTC compared to those diagnosed more than 2 years after delivery (group 1) or before pregnancy (group 3).

Taken together, recent evidence supports the hypothesis that pregnancy may negatively affects the prognosis of DTC. The discrepancy with previous studies could be attributed to the different criteria used for the outcome evaluation, as suggested elsewhere (3). Previous papers used the overall survival, DTC-related death and disease recurrence (evaluated by biopsy or whole body scan) as outcome criteria, which were probably not appropriate for a long survival disease with frequent indolent course. In the present study, according to Vannucchi et al., the persistence/recurrence of disease was investigated using more sensitive and precocious markers such as basal and rhTSH stimulated thyroglobulin and neck ultrasonography, as suggested by European and American guidelines (9, 12).

Nevertheless, the worst outcome in patients of group 2 cannot be referred to a higher prevalence of a worse staging at the time of diagnosis or to a more aggressive histological phenotype because, in our study, no significant differences in the examined clinical and morphological parameters were observed.

The mechanisms by which pregnancy could affect the DTC outcome are not easily explainable. In order to verify whether molecular and/or phenotypical features were influencing the results above, we tested the protein expression of sex hormone receptors, as well as the gene expression of NIS and the prevalence of BRAF mutations in the three groups. Indeed, we cannot support the negative prognostic role of estrogens, as previously

suggested (7), considering that our results did not show any significant expression of ER α and no differences among the three groups were observed. The discrepancy between these results has to be clarified, but a difference in the methodological approach could be considered. For example different antibody dilutions were used in the two works (1:300 vs 1:100 dilution). However, it has to be noted that the good correlation between the low expression of ER α and PGR justifies the reliability of our findings. The immunohistochemical analysis was performed also for the detection of ER β , showing a variable expression without any significant difference among the three groups of patients. Furthermore, aromatase expression resulted generally very low, leading us to keep out its potential pathophysiological role.

In the multivariable logistic regression analysis, BRAF^{V600E} mutations are associated with a worse prognosis, but their similar distribution among the groups excludes a pathophysiological role on the poorer outcome of group 2 patients .

We hypothesized that the worse outcome of group 2 could be explained by a lower response to radioiodine therapy. As resulted by the multivariable logistic regression analysis, NIS lower expression is associated with a higher persistence/recurrence of DTC, but its distribution was not different among the three groups, excluding a role in affecting the outcome of group 2.

In conclusion, our results, obtained in a large homogeneous population, confirm that pregnancy could really exert a negative prognostic role, at least in terms of risk of persistent disease or recurrence, in patients with differentiated thyroid cancer. Further studies are needed to clarify the pathophysiological mechanisms. At the present state of our knowledge, a more careful follow up is needed when diagnosis of DTC occurs during pregnancy or shortly after. However, the impact on DTC prognosis is not so heavy to justify the reconsideration of the American guidelines for the management of thyroid cancer during pregnancy (19).

348

349 **DECLARATION OF INTERESTS**

350 The authors declare that there is no conflict of interest that could be perceived as
351 prejudicing the impartiality of the research reported.

352

353 **FUNDING**

354 This work was partially supported by a grant from the "Fondazione Berlucci" (Brescia,
355 Italy, call year 2011, to MV).

356

357 **AUTHOR CONTRIBUTIONS**

358 Ilaria Messuti and Stefania Corvisieri equally contributed to the drafting of this work.

359

360 **ACKNOWLEDGMENTS**

361 The authors would like to warmly thank dr Claudia Cavallari and dr Marco Tampellini for
362 helpful discussion, suggestions and comments.

363

364

365 **REFERENCES**

366 1) Mazzaferri EL, Robbins RJ, Spencer A , Braverman LE, Pacini F, Wartofsky L et
367 al. A consensus report of the role of serum thyroglobulin as a monitoring method
368 for low-risk patients with papillary thyroid carcinoma *Journal of Clinical*
369 *Endocrinology and Metabolism* 2003 **88** 1433-1441

370 2) Durante C, Montesano T, Torlontano M, Attard M, Monzani F, Tumino S, Costante
371 G, Meringolo D, Bruno R, Trulli F, Massa M, Maniglia A, D'Apollo R, Giacomelli L,

Ronga G, Filetti S; PTC Study Group. "Papillary thyroid cancer: time course of recurrences during postsurgery surveillance." *Journal of Clinical Endocrinology and Metabolism* 2013 **98(2)** 636-642

3) Gustavo Vasconcelos Alves, Ana Paula Santin, and Tania Weber Furlanetto. "Prognosis of Thyroid Cancer Related to Pregnancy: A Systematic Review." *SAGE-Hindawi Access to Research Journal of Thyroid Research* 2011

4) M. Moosa and E. L. Mazzaferri, "Outcome of differentiated thyroid cancer diagnosed in pregnant women," *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 2862–2866

5) S. Yasmeen, R. Cress, P. S. Romano et al., "Thyroid cancer in pregnancy," *International Journal of Gynecology and Obstetrics* 2005 **91** 15–20

6) F. S. Herzon, D. M. Morris, M. N. Segal, G. Rauch, and T. Parnell, "Coexistent thyroid cancer and pregnancy," *Archives of Otolaryngology—Head and Neck Surgery* 1994 **120** 1191–1193

7) G. Vannucchi, M. Perrino, S. Rossi et al., "Clinical and molecular features of differentiated thyroid cancer diagnosed during pregnancy," *European Journal of Endocrinology* 2010 **162** 145–151

8) Magri F, Capelli V, Rotondi M, Leporati P, La Manna L, Ruggiero R, Malovini A, Bellazzi R, Villani L, Chiovato L. "[Expression of estrogen and androgen receptors in differentiated thyroid cancer: an additional criterion to assess the patient's risk](#)" *Endocrine Related Cancer* 2012 18;19(4) 463-71

9) Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, Wiersinga W & European Thyroid Cancer Taskforce. "European consensus for the management of patients

with differentiated thyroid carcinoma of the follicular epithelium. *European Journal of Endocrinology* 2006 **154** 787–803

10)Hedinger C. Histological Typing of Thyroid Tumors: WHO International Histological Classification of Tumors, edn 2. Berlin-Heidelberg-New York: Springer-Verlag, 1988.

11)UICC TNM. Classification of Malignant Tumours; 6th Edition, New York: Wiley Liss 2002

12)Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Sherman SI & Tuttle RM. Management guidelines for patients with thyroid nodules and differentiated thyroid cancer” *Thyroid* 2006 **16** 109–142.

13)Chiovato L, Latrofa F, Braverman LE, Pacini F, Capezzone M, Masserini L, Grasso L & Pinchera A. “Disappearance of humoral thyroid autoimmunity after complete removal of thyroid antigens”. *Annals of Internal Medicine* 2003 **2;139 (5 Pt 1)** 346-351.

14) Spencer CA, Takeuchi M, Kazarosyan M, Wang CC, Guttler RB, Singer PA, Fatemi S, LoPresti JS & Nicoloff JT. “Serum thyroglobulinautoantibodies: prevalence, influence on serum thyroglobulin measurement, and prognostic significance in patients with differentiated thyroid carcinoma.” *Journal of Clinical Endocrinology and Metabolism* 1998 **83(4)** 1121-1127.

15) Volante M, Rapa I, Gandhi M, Bussolati G, Giachino D, Papotti M, Nikiforov YE. “RAS mutations are the predominant molecular alteration in poorly differentiated thyroid carcinomas and bear prognostic impact.” *Journal of Clinical Endocrinology and Metabolism* 2009 **94(12)** 4735-4741.

- 16) Ceppi P, Volante M, Novello S, Rapa I, Danenberg KD, Danenberg PV, Cambieri A, Selvaggi G, Saviozzi S, Calogero R, Papotti M, Scagliotti GV. "ERCC1 and RRM1 gene expressions but not EGFR are predictive of shorter survival in advanced non-small-cell lung cancer treated with cisplatin and gemcitabine." *Annals of oncology* 2006 **17(12)** 1818-1825.
- 17) Richard C. Webb, Robin S. Howard, Alexander Stojadinovic, David Y. Gaitonde, Mark K. Wallace, Jehanara Ahmed, and Henry B. Burch. "The Utility of Serum Thyroglobulin Measurement at the Time of Remnant Ablation for Predicting Disease-Free Status in Patients with Differentiated Thyroid Cancer: A Meta-Analysis Involving 3947 Patients". *Journal of Clinical Endocrinology and Metabolism*, August 2012 **97(8)** 2754-2763.
- 18) [Kim TH](#), [Park YJ](#), [Lim JA](#), [Ahn HY](#), [Lee EK](#), [Lee YJ](#), [Kim KW](#), [Hahn SK](#), [Youn YK](#), [Kim KH](#), [Cho BY](#), [Park do J](#). "The association of the BRAF(V600E) mutation with prognostic factors and poor clinical outcome in papillary thyroid cancer: a meta-analysis". *Cancer* 2012 **1;118(7)** 1764-1773
- 19) Alex Stagnaro-Green, Marcos Abalovich, Erik Alexander, Fereidoun Azizi, Jorge Mestman, Roberto Negro, Angelita Nixon, Elizabeth N. Pearce, Offie P. Soldin, Scott Sullivan, and Wilmar Wiersinga, "Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and Postpartum", *Thyroid* 2011 **21** 1081-1125

FIGURE LEGENDS

Figure 1. Immunohistochemical analysis of hormone receptors. A case of multifocal papillary carcinoma (group 3) (**a**; H&E, original magnification 40x), with the predominant nodule of the follicular variant (**b**; H&E, original magnification 200x), and high expression

of ER α (c), ER β (d) and PGR (e) (c-d-e: immunoperoxidase, original magnification 200x);
 f: scatter plot graphs of the distribution of hormone receptors in the three groups of DTC.

TABLES

Table 1. Clinical, histological and molecular characteristics of patients with a DTC
 diagnosis at least 2 years after delivery (Group 1), during pregnancy or within two years
 after delivery (Group 2), or before pregnancy/nulliparous (Group 3)

* Group 2 was significantly different as compared to both group 1 and group 3.

** The ablation-HTG cut-off was defined according to Webb et al. (17)

Table 2: Logistic regression analysis for persistence/recurrence of DTC

Table 1

	Group 1	Group 2	Group 3	P value
Age at diagnosis (years): Median (range)	40 (25-45)	35 (26-41)	30 (15-45)	<0.001
Duration of follow up (years): Median (range)	5 (1-27)	6 (1-10)	6 (1-20)	0.31
Remission	150/152 (98.7%)	34/38 (89.5%)	143/150(95.3%)	0,023*
Persistence/recurrence	2/152 (1.3%)	4/38 (10.5%)	7/15 (4.7%)	
Number of treatments	1,19	1,21	1,28	0,22

(average)				
Ablation-HTG <10 ng/ml **	127/152 (83,5%)	27/38 (71%)	110/150 (73,3%)	0,060
Ablation-HTG >10 ng/ml **	25/152 (16,5%)	11/38 (29%)	40/150 (26,7)	
High Risk	68/152 (44.7%)	19/38 (50%)	79/150 (52,7%)	0,38
Low Risk	84/152 (55.3%)	19/38 (50%)	71/150 (47,3%)	
TNM (T<3)	105/152 (69%)	26/38 (68.4%)	95/150 (63.3%)	0,85
TNM (T>3)	47/152 (31%)	12/38 (31.6%)	55/150 (36.7%)	
TNM (N -)	114/152 (75%)	29/38 (76.3%)	102/150 (68%)	0,54
TNM (N +)	38/152 (25%)	9/38 (23.6%)	48/150 (32%)	
Histology (High Risk)	54/152 (35.5%)	16/38 (42.1%)	62/150 (41.3%)	0,85
Histology (Low Risk)	98/152 (64.5%)	22/38 (57.9%)	88/150 (58.7%)	
ER α tumor expression	3/12 (25 %)	3/10 (30 %)	4/14 (28.6 %)	0.96
ER β tumor expression	5/12 (41.7%)	5/10 (50%)	7/15 (46.7%)	0.92
PGR tumor expression	4/12 (33.3%)	3/10 (30%)	8/15 (53.3%)	0.419
BRAF V600E mutation	3/12 (25%)	4/9 (44.4%)	9/15 (60%)	0,19
NIS fold change <1	8/12 (66,6%)	5/8 (62,5%)	9/13 (69,2%)	0,9

Table 2

Variable	RR (95% CI)	P value
Pregnancy	1.26 (0.97-1.55)	0.09
Er β positive staining	0.69 (0.38-1.00)	0.06

Presence of BRAF mutation	1.46 (1.16-1.77)	0.005
High NIS expression	0.66 (0.36-0.96)	0.03

464

465